DATA EVALUATION RECORD

PICOXYSTROBIN (ZA1963)

Study Type: OPPTS 870.3100 [§82-1a], Subchronic Oral Toxicity Study in Rats

Work Assignment No. 7-1-256 A (MRID 48073731)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity - rat; OPPTS 870.3100 [§82-1a]; OECD 408.

PC CODE: 129200 **DP BARCODE:** D378236
TXR #: 0056696 **SUBMISSION #:** S873059

TEST MATERIAL (PURITY): Picoxystrobin (93.3% a.i.)

SYNONYMS: ZA1963; methyl (α*E*)-α-(methoxymethylene)-2-[[[6-(trifluoromethyl)-2-pyridinyl]oxy]methyl]benzeneacetate

CITATION: Rattray, N.J. (1999) ZA1963: 90 day feeding study in rats. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Report No.: CTL/P/5088, March 11, 1999. MRID 48073731. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 48073731), picoxystrobin (ZA1963; 93.3% a.i.; Batch No. P25) was administered in the diet to Wistar rats (12/sex/dose) at doses of 0, 100, 500, or 1250 ppm (equivalent to 0/0, 8.5/9.7, 41.7/48.1, and 104.9/120.1 mg/kg/day for males/females) for 13 weeks.

No adverse, treatment-related effects were observed on mortality, clinical signs, ophthalmoscopic examination, hematology, clinical chemistry, urinalysis, organ weights, or gross or microscopic pathology.

At 1250 ppm, decreased ($p<=0.05$) body weights were observed throughout the study in males (decr. 7-10%) and females (decr. 5-9%). Body weight gain during the initial week of treatment was decreased by 27-28%, and overall (Weeks 1-14) body weight gains were decreased by 14-15%. Decreased food consumption was observed each week during Weeks 1-10 in males (decr. 7-15%) and during Weeks 3-7 and 10 in females (10-15%). Food utilization was decreased during Weeks 1-4 in the females (11.9% treated vs 14.1% controls).

The LOAEL is 1250 ppm (104.9 mg/kg/day in males/females) based on decreased body weight, body weight gains and food consumption. The NOAEL is 500 ppm (41.7 mg/kg/day in males/females).

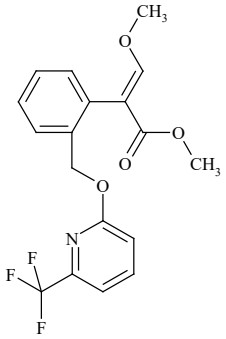
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100; OECD 408) for a subchronic oral toxicity study in the rat.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Picoxystrobin

Description:	Pale yellow solid
Batch No.:	P25
Purity (w/w):	93.3% a.i.
Stability of compound:	The test compound was stable in the dietary formulations for at least 89 days at room temperature.
CAS #:	117428-22-5
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat
Strain:	Alpk:AP$_f$SD
Age and group mean weight at study initiation:	Approximately 5 weeks old; 153.7-154.7 g males; 131.7-134.4 g females
Source:	Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, England
Housing:	4 per cage (same sex) in multiple rat racks
Diet:	CTL diet (Special Diets Services Limited, Stepfield, Witham, Essex, UK), *ad libitum*, except during urine collection
Water:	Tap water, *ad libitum*, except during urine collection
Environmental conditions	
Temperature:	21±2ºC
Humidity:	40-70%
Air changes:	At least 15/hour
Photoperiod:	12 Hours light/12 hours dark
Acclimation period:	Approximately 2 weeks

B. STUDY DESIGN

1. In life dates: Start: November 14, 1995; End: Approximately February 13, 1996

2. Animal assignment/dose levels: The animals were randomly assigned to the test groups shown in Table 1.

TABLE 1: Study design [a]			
Test group	**Dose to animal (ppm)**	**Compound intake (mg/kg/day in M/F)**	**No. rats/sex killed at Week 13**
Control	0	0/0	12
Low	100	8.5/9.7	12
Mid	500	41.7/48.1	12
High	1250	104.9/120.1	12

a Data were obtained from pages 18 and 95 of MRID 48073731.

3. **Dose-selection rationale:** The Sponsor stated that the dose levels selected for this study were based on the results of a preliminary dose range finding study in the Alpk:AP_fSD rat carried out in this laboratory.

4. **Dose preparation and analysis:** The test compound was mixed with the diet to form a pre-mix, which was further diluted with appropriate amounts of the diet to achieve the desired concentrations. The frequency of preparation was not reported. Test compound stability in the diet was evaluated in the 100 and 1250 ppm dietary formulation for up to 89 days at room temperature. Homogeneity (top, middle, and bottom strata) was also evaluated in the 100 and 1250 ppm dietary formulations. Concentrations were measured twice during the study for each dietary concentration.

Results

Homogeneity (% coefficient of variation): 2.45-3.72%

Stability (% of initial): 93.5-100.0%

Concentration (% of nominal): 96.4-103.8%

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** All data were evaluated using the GLM procedure in SAS, separately for males and females. The following statistical analyses were performed.

PARAMETER	STATISTICAL ANALYSES
Body weight	Body weights were considered by analysis of covariance on initial (week 1) body weight. [a]
Food consumption Food utilization Hematology Clinical chemistry Urinalysis	Analysis of variance was conducted. [a] For hematology, clinical chemistry, and urinalysis, male and female data were also analyzed together and the results examined to determine whether any differences between control and treated groups were consistent between sexes.
Organ weights	Analysis of variance and analysis of covariance on final body weight were performed. [a]

a Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

These statistical analyses were considered appropriate.

C. METHODS

1. Observations

a. Cageside observations: All animals were checked daily for changes in clinical condition or behavior.

b. Clinical examinations: Detailed clinical observations were performed weekly.

c. Neurological evaluations: Neurological evaluations were not conducted in this study; however, acute and subchronic neurotoxicity studies in rats were concurrently submitted (MRIDs 48073753 and 48073752, respectively).

2. Body weight and body weight gain: All animals were weighed prior to treatment, weekly until scheduled termination, and at necropsy.

3. Food consumption, food utilization, and compound intake: Food consumption (g/rat/day) was measured for each cage and calculated each week. Food utilization per cage (g body weight gain/100 g food) was calculated for Weeks 1-4, 5-8, 9-13, and 1-13. At weekly intervals, compound intake (mg/kg/day) was calculated from the group mean bodyweight and food consumption data, and overall compound intake was reported (Table 1).

4. Ophthalmoscopic examination: The eyes of all animals were examined prior to treatment and the control and 1250 ppm groups were examined during the week prior to termination.

5. Hematology and clinical chemistry: At termination, all surviving rats were bled by cardiac puncture, and blood samples were collected for hematology and clinical chemistry analyses. The following CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*		Reticulocyte count (RET)
	Blood clotting measurements*	X	Blood cell morphology
X	(Activated partial thromboplastin time)	X	Red cell distribution width
	(Fibrinogen concentration)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium (CA)	X	Albumin* (ALB)
X	Chloride (CL)	X	Creatinine*
	Magnesium	X	Urea nitrogen* (BUN)
X	Phosphorus, inorganic (P)	X	Total cholesterol* (CHO)
X	Potassium* (K)		Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein (PRO)*
	Cholinesterase (CHE) [a]	X	Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Albumin : globulin (A/G)
X	Alanine aminotransferase (ALT/also GPT)* [a]		
X	Aspartate aminotransferase (AST/also GOT)* [a]		
	Sorbitol dehydrogenase*		
X	Gamma-glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. Urinalysis: Individual urine samples were collected from all rats during the week prior to termination. Samples were collected over a period of 16-18 hours during which the rats were housed individually in metabolism cages without food or water. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
	Sediment (microscopic)		Nitrate
X	Protein*		Urobilinogen

* Optional for 90-day oral rodent studies

7. Sacrifice and pathology: Animals were euthanized by exsanguinations under terminal anesthesia induced by halothane Ph. Eur. vapor. The following CHECKED (X) tissues were collected and examined microscopically (except as noted). Additionally, the (XX) organs were weighed, paired organs weighed together.

	DIGESTIVE SYSTEM
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall bladder (not rat)*
	Bile duct (rat)
X	Pancreas*
	RESPIRATORY
X	Trachea*
X	Lung*
X	Nose* [a]
X	Pharynx* [a]
X	Larynx* [a]

	CARDIOVASC./HEMAT.
X	Aorta*
X	Heart*+
X	Bone marrow*
X	Lymph nodes*
X	Spleen*+
X	Thymus*+
	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
X	Prostate*
X	Seminal vesicles*
X	Ovaries*+
X	Uterus*+
X	Mammary gland* (females)
X	Cervix

	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve (sciatic)*
X	Spinal cord*
X	Pituitary*
X	Eyes (retina, optic nerve)*
	GLANDULAR
XX	Adrenal gland*+
X	Lacrimal/Harderian gland
X	Parathyroid*
X	Thyroid*
	OTHER
X	Bone (femur and sternum)
X	Skeletal muscle
X	Skin*
X	Knee joint
X	All gross lesions and masses*

a The oral and nasopharyngeal cavities were stored.
* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.

The Sponsor stated that all tissues were fixed in an appropriate fixative. All tissues (except the oral and nasopharyngeal cavities) from the control and 1250 ppm groups and decedents were routinely processed, stained with hematoxylin and eosin, and examined microscopically.

II. RESULTS

A. OBSERVATIONS

1. Mortality: One 1250 ppm male was terminated for humane reasons during Week 7. This death was not considered related to treatment.

2. Clinical signs of toxicity: No treatment-related effect was noted on clinical signs. In the 500 and 1250 ppm females, dry sores were observed (3-4/12 affected vs 0/12 controls), as well as a blackened tip of tail (3-4/12 affected vs 0/12 controls). These findings were considered incidental.

B. BODY WEIGHT AND BODY WEIGHT GAIN: At 1250 ppm, decreased ($p \leq 0.05$) body weights were observed throughout the study in males (↓7-10%) and females (↓5-9%; Table

2). Body weight gain during the initial week of treatment was decreased by 27-28%, and overall (Weeks 1-14) body weight gains were decreased by 14-15%. Body weight and body weight gains in the other treated groups were similar to controls.

TABLE 2. Body weights and body weight gains (g) in rats treated with Picoxystrobin in the diet for 13 weeks [a]

Week (s)	Dose (ppm)			
	0	100	500	1250
Males				
1 [b]	154.4±10.4	153.9±10.2	154.7±12.4	153.7±11.4
2 [c]	206.4	208.3	204.7	192.0** (↓7)
14 [c]	495.5	484.7	478.2	447.8** (↓10)
Weeks 1-2 [d]	52.4	54.1	50.6	37.6 (↓28)
Weeks 2-7	182.6	176.5	172.9	157.4 (↓14)
Weeks 7-14	107.0	100.1	101.4	98.0 (↓8)
Weeks 1-14	342.0	330.7	324.9	293.0 (↓14)
Females				
1 [b]	131.7±9.5	132.9±6.6	134.4±10.6	134.1±10.1
2 [c]	158.8	159.0	158.9	151.6** (↓5)
5 [c]	218.3	218.8	217.1	198.6** (↓9)
14 [c]	274.2	277.6	268.5	251.8** (↓8)
Weeks 1-2 [d]	25.3	25.7	25.8	18.4 (↓27)
Weeks 2-7	77.5	77.2	71.1	65.5 (↓15)
Weeks 7-14	37.4	41.3	38.8	35.0 (↓6)
Weeks 1-14	140.2	144.2	135.7	118.9 (↓15)

a Data (n=11-12) were obtained from Table 7 on pages 51-54 in MRID 48073731. Percent difference from controls is included in parentheses, and was calculated by the reviewers.
b Mean weights ± SD
c Adjusted (covariable is Week 1) mean weights
d Body weight gains were calculated by reviewers from mean body weights reported in the cited data. Statistical analyses were not performed.
** Significantly different ($p \leq 0.01$) from the control groups

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** At 1250 ppm, decreased food consumption was observed each week during Weeks 1-10 in males (↓7-15%) and during Weeks 3-7 and 10 in females (10-15%; Table 3). Minor, transient decreases in food consumption were noted in the 500 ppm males, and food consumption in the other treated groups was similar to controls.

2. **Food utilization:** Food utilization was decreased ($p \leq 0.05$) during Weeks 1-4 in the 1250 ppm females (11.9% treated vs. 14.1% controls) (Table 4). Food utilizations were comparable among the control groups and other treated groups during the rest of the test periods.

TABLE 3. Mean (±SD) food consumption (g/rat/day) in rats treated with Picoxystrobin in the diet for 13 weeks [a]

Week (s)	Dose (ppm)			
	0	100	500	1250
Males				
1	26.0±0.7	25.9±0.4	25.2±0.8	22.0±1.2** (↓15)
10	30.8±1.6	29.9±0.8	29.1±0.8	28.7±1.7* (↓7)
13	28.0±1.4	27.9±0.5	27.0±0.3	26.2±1.6
Females				
1	20.3±0.9	20.5±0.0	19.7±0.9	18.9±1.2
3	21.8±1.3	21.9±0.1	21.5±0.4	19.6±0.7* (↓10)
5	22.7±0.3	21.9±0.4	20.7±1.3	19.3±2.0* (↓15)
13	20.3±0.9	20.4±0.5	20.6±1.4	19.2±1.1

a Data (n=3 cages; each cage contained 4 rats) were obtained from Table 8 on pages 55-56 in MRID 48073731. Percent difference from controls is included in parentheses, and was calculated by the reviewers.
* Significantly different ($p \leq 0.05$) from the control groups
** Significantly different ($p \leq 0.01$) from the control groups

Table 4. Food Utilization (gm growth/100 gm food)

Week s	Concentration (ppm)			
	0	100	500	1250
	Males			
1-4	22.92 ± 1.11	22.56 ± 0.21	22.36 ± 1.21	21.50 ± 2.26
5-9	10.67 ± 0.40	10.50 ± 0.46	11.01 ± 0.71	10.69 ± 0.64
9-13	6.30 ± 0.19	5.84 ± 0.33	6.06 ± 0.85	5.86 ± 0.62
Overall (wk 1-13)	12.59 ± 0.40	12.30 ± 0.02	12.46 ± 0.64	11.90 ± 0.93
	Females			
1-4	14.11 ± 1.04	14.35 ± 0.61	14.44 ± 0.67	11.91 ± 0.98*
5-9	4.90 ± 0.63	5.62 ± 0.32	4.66 ± 0.91	5.91 ± 0.79
9-13	3.16 ± 0.55	3.10 ± 1.02	3.13 ± 0.56	2.97 ± 0.58
Overall (wk 1-13)	6.98 ± 0.64	7.27 ± 0.61	7.01 ± 0.47	6.53 ± 0.16

Data excerpted from the report: p.57; N=3 cages, each cage contained 4 rats. *: Statistically significant ($p<0.05$)

3. **Compound consumption:** Compound intake values (mg/kg/day) are presented in Table 1 of this DER.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related effects were noted during the ophthalmoscopic examinations.

E. BLOOD ANALYSES

1. **Hematology:** No adverse, treatment-related effects were noted on hematological parameters. In the 1250 ppm females, white blood cell, neutrophil, lymphocyte, and monocyte counts were increased ($p \leq 0.05$) by 21-42%. However, when the results of one animal (#91) were removed from the statistical analysis, the values in the 1250 ppm females were similar to controls. All other differences ($p \leq 0.05$) in the treated groups compared to the controls were minor.

2. **Clinical chemistry:** Alkaline phosphatase was decreased in the 500 and 1250 ppm males (↓23-30%) and in the 1250 ppm females (↓22%; Table 4). Total bilirubin was increased in the 1250 ppm females (↑30%). Other differences were minor. In the absence of histological

finding and other changes in other parameters, the alkaline phosphatase and bilirubin changes were not considered to be treatment-related.

TABLE 4. Mean (±SD) of selected plasma clinical chemistry parameters in rats treated with Picoxystrobin in the diet for 13 weeks [a]

Parameter	Dose (ppm)			
	0	100	500	1250
Males				
Alkaline phosphatase (IU/L)	206±52	193±25	159±26** (↓23)	144±13** (↓30)
Females				
Alkaline phosphatase (IU/L)	113±28	112±20	103±13	88±12* (↓22)
Total bilirubin (µmol/L)	1.67±0.65	1.67±0.49	2.00±0.43	2.17±0.39** (↑30)

a Data (n=11-12) were obtained from Table 11 on pages 63-66 in MRID 48073731. Percent difference from controls is included in parentheses, and was calculated by the reviewers.
* Significantly different ($p \leq 0.05$) from the control groups
** Significantly different ($p \leq 0.01$) from the control groups

F. URINALYSIS: No adverse, treatment-related effects were noted on urinalysis parameters. Minor increases were noted in the urinary pH of the 500 and 1250 ppm females. A very few urinary crystals were noted in the 1250 ppm females (4 rats) compared to controls (1 rat). Other urinalysis parameters in the treated groups were similar to controls.

G. SACRIFICE AND PATHOLOGY

1. Organ weight: No adverse, treatment-related effects were noted on organ weights. Only minor differences were observed.

2. Gross pathology: No treatment-related effect was observed during necropsy.

3. Microscopic pathology: No treatment-related effect was observed during histology.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: At 1250 ppm, decreases in body weight, food consumption, and food utilization were observed. No adverse effects were observed at 500 ppm. The LOAEL is 1250 ppm, and the NOAEL is 500 ppm.

B. REVIEWER'S COMMENTS: No adverse, treatment-related effects were observed on mortality, clinical signs, ophthalmoscopic examination, hematology, clinical chemistry, urinalysis, organ weights, or gross or microscopic pathology.

At 1250 ppm, decreased ($p <= 0.05$) body weights were observed throughout the study in males (↓7-10%) and females (↓5-9%). Body weight gain during the initial week of treatment was decreased by 27-28%, and overall (Weeks 1-14) body weight gains were decreased by 14-15%. Decreased food consumption was observed each week during Weeks 1-10 in males (↓7-15%) and during Weeks 3-7 and 10 in females (↓10-15%). Food utilization was decreased during Weeks 1-4 in the females.

The LOAEL is 1250 ppm (120.1 mg/kg/day in males/females) based on decreased body weight, body weight gains, and food consumption. The NOAEL is 500 ppm (41.7 mg/kg/day in males/females).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100; OECD 408) for a subchronic oral toxicity study in the rat.

C. **STUDY DEFICIENCIES:** The heart, spleen, thymus, ovaries, and uterus were not weighed, but this was considered a minor deficiency that does not affect the conclusions of this review.